

Chapter 6 Application of beneficial microorganisms rhizobacteria to improve plant production in protected natural areas

Capítulo 6 Aplicación de microorganismos benéficos rizobacterias para mejorar la producción vegetal en espacios naturales protegidos

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Abstract

Protected Natural Areas generate environmental services, form soil, are habitats of wide biodiversity of plants, animals, insects and microorganisms, recharge of aquifers, capture of CO₂, buffer the effects of global climate change. They provide economic resources for the communities that live in the areas. Numerous actions have been generated to maintain the conservation of biological diversity. Through comprehensive strategies and actions, the impact of damage caused by anthropocentric activities can be reduced. Forest soils have an enormous variety of living forms that obtain their energy mainly from forms of organic matter derived from plants and animals. The major biological component of forest soils are the roots of plants, microorganisms and animals in the soil. A group of beneficial bacteria that inhabit the rhizosphere region receive organic acids from plants, and the bacteria provide plant protection and better nutrient uptake. Among the benefits that bacteria provide directly are: nitrogen fixation, phosphorus solubilization, production of phytohormones such as: auxins, gibberellins, indole acetic acid. In the state of Guanajuato, the works that have been carried out with isolated rhizobacteria from the soil are from several Protected Natural Areas and guava orchards. Rhizobacteria isolates have been tested on fruit, food and ornamental plants.

Soil, Forest, Conservation, Bacteria

Resumen

Las Áreas Naturales Protegidas generan servicios ambientales, forman suelo, son hábitats de amplia biodiversidad de plantas, animales, insectos y microorganismos, recarga de acuíferos, captura de CO₂, amortiguan los efectos del cambio climático global. Proporcionan recursos económicos a las comunidades que viven en las zonas. Se han generado numerosas acciones para mantener la conservación de la diversidad biológica. Mediante estrategias y acciones integrales, se puede reducir el impacto de los daños causados por las actividades antropocéntricas. Los suelos forestales tienen una enorme variedad de formas vivas que obtienen su energía principalmente de formas de materia orgánica derivadas de plantas y animales. El principal componente biológico de los suelos forestales son las raíces de las plantas, los microorganismos y los animales del suelo. Un grupo de bacterias beneficiosas que habitan en la región de la rizosfera reciben los ácidos orgánicos de las plantas, y las bacterias proporcionan protección a las plantas y una mejor absorción de nutrientes. Entre los beneficios que las bacterias proporcionan directamente están: fijación de nitrógeno, solubilización de fósforo, producción de fitohormonas como: auxinas, giberelinas, ácido indol acético. En el estado de Guanajuato, los trabajos que se han realizado con rizobacterias aisladas del suelo son de varias Áreas Naturales Protegidas y huertas de guayaba. Los aislamientos de rizobacterias se han probado en plantas frutales, alimenticias y ornamentales.

Suelo, Bosque, Conservación, Bacterias

1 Introduction

Forests and Protected Natural Areas are essential ecosystems for life. Many cultures have relied on the products obtained from the forest: wood as fuel or in construction, charcoal, hunting animals, resins, fruits, medicinal plants, etc. In the Mexican territory, at the federal and state level, territorial and aquatic spaces have been designated for conservation, the Protected Natural Areas (PNA) are defined as the areas of the territory where the original environments have not been significantly altered by the activity of the being. human beings either require preservation or restoration. The aim is to conserve the biodiversity of the different ecosystems. The PNAs are regulated by the General Law of Ecological Balance and Environmental Protection with protection, conservation, restoration and development regimes. PNAs generate environmental services, form soil, are habitats of wide biodiversity of plants, animals, insects and microorganisms, recharge of aquifers, capture of CO₂, buffer the effects of global climate change.

They provide economic resources for the communities that live in the areas. There is concern about the proper use of natural resources and to achieve this, numerous actions have been generated to maintain the conservation of biological diversity. Through comprehensive strategies and actions, the impact of damage caused by anthropocentric activities such as deforestation, fires, charcoal production, livestock, agriculture, wildlife hunting, expansion of cities, extraction of soil and litter for sale as potting soil, extraction of plant and animal specimens in endangered cases, among others.

A natural resource within the PNA is the soil, a system of inorganic and organic components and living organisms and microorganisms. In the soil, all the vegetation of the natural areas develops, in which the plants obtain mechanical support, water and nutrients. The soil is a very dynamic system, in which a great variety of biochemical reactions are carried out, facilitated by the microbial communities that inhabit it, the formation of the soil itself and its organic matter, the recycling of nutrients, etc.

A group of beneficial bacteria that inhabit the rhizosphere region receive organic acids from plants and the bacteria provide plant protection and better nutrient uptake. Among the benefits that bacteria provide directly are: nitrogen fixation, phosphorus solubilization, production of phytohormones such as: auxins, gibberellins, indole acetic acid. Other benefits of the plant-rhizobacteria interaction is protection against phytopathogenic microorganisms by the production of toxic compounds that stop the advance of the phytopathogen.

Importance of protected natural areas

The Protected Natural Areas are the areas of the state territory where the original environments have not been significantly altered by human activity or need to be preserved or restored, and in the specific case of those classified as areas of sustainable use, it has for The objective of producing goods and services that respond to the economic, social and cultural needs of the population, based on the sustainable use of compatible uses, being located in areas that include hydrological basins, forest resources and elements of wild flora and fauna, in the that there are agricultural developments, recreational potential and rural populations (Institute of Ecology of the State of Guanajuato, 2015).

The geological characteristics of the area are basalt for the most part, combined with zones of volcanic breccia, both extrusive igneous rocks. The highest parts of the hills present Haplic Faeozem-type soils, easy to erode when found on slopes and slopes, and the remaining surface is dominated by vertisol pelic soils with a stony phase (Instituto de Ecología del Estado de Guanajuato, 2004).

The Guanajuato, Mexico state is located between the parallels 19° 55 '08' 'and 21° 52' 09' " of north latitude and the meridians 99° 41 '06' 'and 102° 09' 07' " of west longitude. The total surface of the state is 30,589 km, see in figure 6.1. The predominant vegetation of the PNA is oak forest (*Quercus deserticola*), tropical deciduous forest and crasicaule scrub. In The Gavia it is represented mainly by trees of the *Bursera* genus and other species such as *Ipomoea spp.* and *Acacia spp.* The crasicaule scrub in The Culiacán hill is represented by species with succulent stems, associated with spiny-type species, herbaceous elements and some kinds of grasses, while in Gavia there are *Opuntia*, *Myrtillocactus*, *Mimosa spp.*, *Acacia spp.*, and *Ipomoea spp.* (Institute of Ecology of the State of Guanajuato, 2004) see in figure 6.2.

Figure 6.1 Map of Protected Natural Areas in the state of Guanajuato, Institute of Ecology of the State of Guanajuato, Mexico

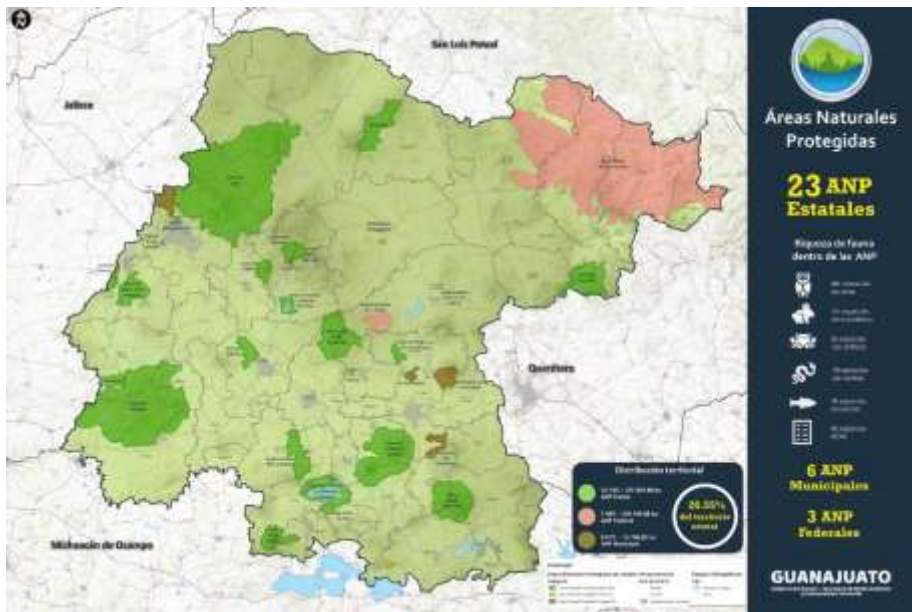
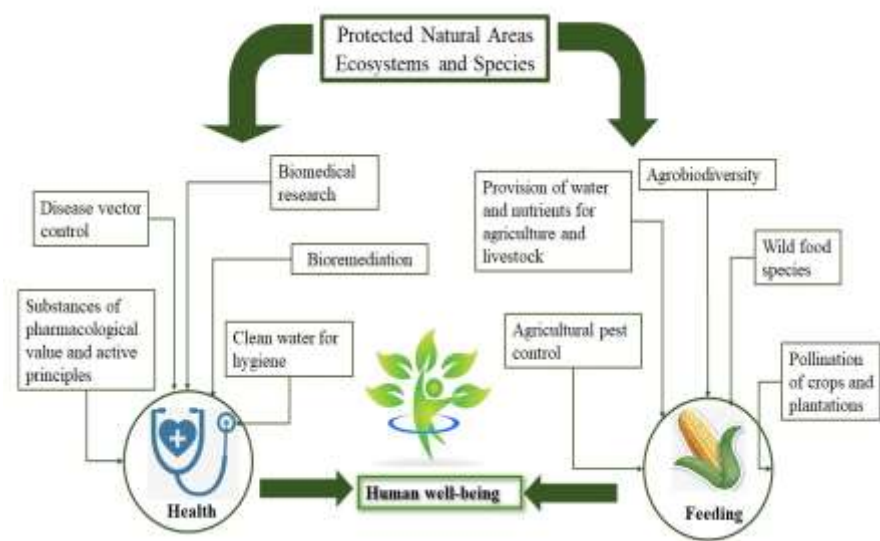


Figure 6.2 Oak forest (*Quercus* spp.) in Protected natural areas in Guanajuato, Mexico



The importance of preserving protected natural areas also lies in the services that these areas provide to humanity, which are observed in the figure 6.3.

Figure 6.3 Services provided by protected natural areas, Secretary of the Environment and Natural Resources and National Commission for Protected Areas (CONANP)



Threats to protected natural áreas

One of the main problems of Cerro del Culiacán and La Gavia is deforestation, caused mainly by changes in land use, use of trees for firewood and handicraft production, extensive cattle ranching, effects by pests and forest fires.

The aquifer resource is extremely scarce and combined with deforestation and loss of soil, it causes an increase in the speed of runoff and a decrease in infiltration and water retention rates; in addition to presenting uncontrolled exploitation of existing material banks in the area (IEE, 2015).

The accelerated destruction of forests by the change of land use for agriculture, livestock, expansion of cities, roads, excessive logging, charcoal production, pests among other forms of soil disturbance. Agrochemical compounds (pesticides, herbicides and fertilizers) can have an adverse effect if they are used in excess, since they can affect the physiology and biochemistry of the soil, reflecting in reduced respiration, enzymatic activity, microbial biomass, nitrogen content and changes in soil microbial diversity (Yang et al., 2000).

Excessive logging is a very common practice in forests and in some it is selective towards some tree species. Deforestation can lead to soil erosion and destatization of the water table, which in turn favors floods or droughts and the loss of biodiversity. The opening of roads and the extraction processes cause the compaction of the soil, making it more susceptible to the loss of nutrients (FAO, 2000, WRI, 2001). Extraction of soil and litter that is sold as potting soil, the sale of these elements leads to more vulnerability to erosion, less water retention, loss of soil organic matter and nutrients, in addition to requiring hundreds to thousands of years for soil formation depending on geochemical, climatic and geographic conditions.

Soil and microbial communities

Forest soils have an enormous variety of living forms that obtain their energy mainly from forms of organic matter derived from plants and animals. The major biological component of forest soils are the roots of plants, microorganisms and animals in the soil. Together these organisms play an important role in the function of forest ecosystems through their participation in the degradation of organic matter. Through these processes there is a positive effect on the availability of nutrients for plant growth and on the structure of the soil.

Soil as an ecosystem is made up of five main components: mineral matter, water, air, organic matter, and living organisms. Soil contains five main groups of microorganisms: bacteria (archaea), actinomycetes, fungi, algae, and protozoa (Alexander, 1980). Bacteria are particularly prominent because there are many populations in a soil and because they are the most abundant group. The role of bacteria in the soil is very varied: they may be participating in the regulation of biogeochemical cycles, in association or interaction with plants, nitrogen fixation, growth promotion, degradation of xenobiotic compounds, recirculation of nutrients, degradation of cellulose, hemicellulose, lignin, polysaccharides, hydrocarbons, proteins, urea, immobilization of organic phosphorus, oxidation of sulfur, formation of organic iron compounds, mobilization of potassium, etc., (León, 1991).

Rhizobacteria

Plant growth-promoting rhizobacteria are a group of bacteria that inhabit the root of plants and the soil attached to it, this space is known as the rhizosphere (Cassán et al., 2009). A variety of organic acids are produced in the rhizosphere that can be metabolized by rhizobacteria. The rhizobacteria in turn provide nutrients from the soil to the plant (Marschner, et al, 2004; Lugtenberg and Kamilova, 2009). This group of bacteria provide benefits to plants through various mechanisms: N₂ fixation, phytohormone production, phosphate solubilization, synthesis of enzymes such as ACC deaminase that reduces ethylene levels, biological control, siderophore production, antibiotics, activation of the induced systemic response and production of lytic enzymes (Glick, 1995; Dobbelaere, et al, 2003; Esquivel et al., 2013). The products generated by the various mechanisms have direct and indirect effects on the plant's development and growth, such as: improvement in germination, greater development of the root, stems, leaves and fruits or defense against phytopathogenic organisms (Glick, 1995; Dobbelaere et al., 2003; Esquivel et al., 2013). Bacteria with ACC deaminase activity are of special interest since these can decrease the level of ethylene in the plant root, due to the degradation of the ethylene precursor, 1-aminocyclopropane -1-carboxylic acid (ACC). Bacteria carry out this process by means of the ACC deaminase enzyme, which by degrading ACC generates ammonium and α -ketobutyrate products, thus the bacteria attached to the root of the plant consume ACC and lower the level of ethylene associated with stress signals and favor root elongation (Glick, et al, 1999, Holgin et al., 2003, Esquivel et al., 2013).

The use of rhizobacteria in plants of agronomic importance has resulted in an important alternative to production systems with a high consumption of fertilizers and agrochemicals (Grageda, et al., 2012; Martínez, et al., 2013), however, research and monitoring of use for plants of forest importance or for recovery and conservation of PNA is much less. In some of these works they found that the benefits of rhizobacteria have been used for reforestation of desert areas (Berreto, et al, 2007; Ogata, et al, 2008). Due to its ability to fix nitrogen, *Azospirillum brasilense* has been used in cardón plants where they achieved improvement in the development of the plant and in the regeneration of the soil (Holguin, et al., 2003). The genera of *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, isolated from the rhizosphere of *Caesalpinia spinosa* "tara" trees were tested on alfalfa (*Medicago sativa*), tara (*Caesalpinia spinosa*), pallar (*Phaseolus lunatus*) and bean (*Phaseolus vulgaris*) seeds and Ogata, et al (2008) observed that the tested strains increase the germination of the mentioned crops.

In another work, strains were isolated from the rhizosphere of *Anacardium excelsum*, which is an arboreal species native to dry forests in Central and South America. The isolates were from *Pseudomonas fluorescens*, *P. putida* and *Bacillus licheniformis* and were used to evaluate germination and growth of *Anacardium excelsum* seedlings, finding positive effects in both processes (Barreto, et al 2007).

Other authors reported the effectiveness in forest restoration, testing growth-promoting rhizobacteria in 11 native species (*Tipuana tipu* (Benth O.) Kuntze (white tipa), *Pterogyne nitens* Tul (*Tipa colorada*), *Aspidosperma quebracho blanco* Schlecht (Quebracho blanco), *Schinopsis lorentzii* (Griseb) Engler (Quebracho colorado santiagueño), *Enterolobium contortisiliquum* (Well) Morong (Pacar, timb), *Jacaranda mimosifolia* D. Don (Jacarand or tarco), *Cedrela lilloi* C. DC. (Cedro coya or hairy cedar), *Caesalpinia paraguariensis* (D. Parodi) Burkart (Guayacn), *Prosopis chilensis* (Mol) Stuntz Chilean Algarrobo, *Juglans australis* griseb. (Nogal criollo), *Anadenanthera colubrina* Griseb (Cebil Colorado) from the forests of Jujuy, Argentina. Here they observed improvements such as the germination, development, establishment in the site to be recovered and plant health, proving that the isolated strains are effective for the purposes (Lzzaro, et al., 2011).

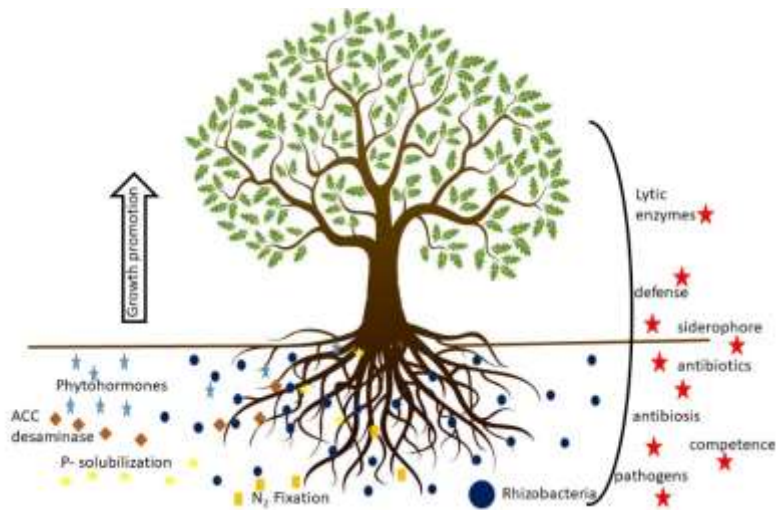
With other purposes, it has begun to use plant growth promoting bacteria as components of environmental improvement for the recovery of soils in degraded arid zones, reintegrating native vegetation with plant growth promoting microorganisms (Garca, et al., 2013).

Biofertilizers made from bacteria

The soil is inhabited by many microorganisms, among them are bacteria that can be free-living in the soil, in soil attached to the roots of plants and in the cells of the roots of plants, forming a symbiosis (association between two organisms with benefits for both). Of all the groups of bacteria that inhabit the soil, the so-called PGPR stands out for its acronym in English (Promoting Grow Plant Rhizobacteria), in Spanish rhizobacteria that promote plant growth, rhizobacteria refers to that inhabit the space called rhizosphere (Cassn et al., 2009), soil space in close contact with plant roots. This type of plant-bacteria interaction occurs in a special way in the rhizosphere because the roots of the plants produce a variety of organic acids that can be metabolized by bacteria and bacteria in turn provide nutrients from the medium to the plant (Marschner, et al., 2004; Lugtenberg, and Kamilova, 2009).

Two mechanisms have been described by which plant growth-promoting bacteria provide a benefit to the plant, which are known as: direct and indirect mechanisms. Among the benefits that bacteria provide directly are: nitrogen fixation, phosphorus solubilization, production of phytohormones such as: auxins, gibberellins, indole acetic acid (Glick 1995; Dobbelaere et al., 2003; Esquivel et al., 2013). On the other hand, the benefits that this group of bacteria provides to plants indirectly through the production of compounds that can be antibiotics, siderophores (a molecule that traps iron), among others, that act on other microorganisms that can cause diseases in plants. plants, these mechanisms are seen in figure 6.4 (Glick 1995; Dobbelaere et al., 2003; Esquivel et al., 2013).

Figure 6.4 Mechanisms of the beneficial effects of rhizobacteria on plants



Applications of rhizobacteria with plants from natural áreas

It is well known that there is a continuous deterioration in environmental quality throughout the planet due to human activities on natural areas, mainly deforestation, burning of waste with excessive release of pollutants, such as heavy metals, extraction of forest soil (potting soil), change in the use of land for agricultural production with excess of agrochemicals and pesticides and grazing areas and animal production. Some of the emerging strategies are the use of technologies that plants use as extractors, mitigators and stabilizers of pollutants (phytoremediation) or that stabilize and recover soil productivity (reforestation) in conjunction with beneficial rhizobacteria and other organisms such as mycorrhizal fungi.

Some plants cannot establish in these degraded systems, and even if they do, they are generally affected by adverse environmental conditions, such as excessive concentration of pollutants, extreme pH (high or low), shortages of nutrients, poor soil structure (organic matter and others) and a severely affected or even eliminated microbial community; As a way to help in its establishment, its inoculation with beneficial rhizobacteria and arbuscular mycorrhizal fungi has been proposed. These microorganisms are currently under experimentation in programs of revegetation, reforestation of eroded soils, biological wastewater treatment, phytoremediation, phytostabilization and restoration of ecosystems (De-Basch, 2013).

The use of rhizobacteria in plants of agronomic importance has resulted in an important alternative to production systems with a high consumption of fertilizers and agrochemicals, thus working towards sustainable agriculture (Orona and Leos, 2020), however, research and monitoring of use for plants of forest importance or for recovery and conservation of PNA is much less. In some of these works they found that the benefits of rhizobacteria have been used for reforestation of desert areas (Berreto et al., 2007). In another work, strains were isolated from the rhizosphere of *Anacardium excelsum*, which is an arboreal species native to dry forests in Central and South America. The quantity and diversity of growth-promoting rhizobacteria has been considered as a biological marker of soil health and quality, comparing a forest and an agricultural soil, taking into account the physicochemical properties of each soil (Flores et al., 2018). In some works, efficient microorganisms have been used to improve plant production, as indicated by Sucapuca (2021) by applying efficient microorganisms in wheat crops in winter season (*Triticum aestivum* L.) and found improvements in forage yield and nutrient content, in addition to continuing to use it, it could reduce costs of production. In another proposal, efficient microorganisms were used in urban orchards for tomato (*Solanum lycopersicum*) production, finding improvement in production by using efficient microorganisms, with the possibility of reducing the application of agrochemicals (Frías, 2021).

In the state of Guanajuato, the works that have been carried out with isolated rhizobacteria from the soil are from various PNAs and from guava orchards. The rhizobacteria isolates were characterized and a collection was generated with gram positive and gram negative bacteria, the majority of the genus *Bacillus* spp. To verify their capacity for growth-promoting rhizobacteria, they were tested with plants of importance for the PNA such as Huizache (*Acacia farneciana*), which is a vegetation of the region and of economic and ethnobotanical importance; Furthermore, guava (*Psidium guajava*), lentil (*Lens culinaris*), cucumber (*Cucumis sativus*), radish (*Raphanus sativus*) have also been tested with plants of nutritional importance; in ornamentals such as marigolds (*Tagetes erecta*) and sunflowers (*Helianthus annuus*). In all cases there have been beneficial effects from the germination of the seeds and their development. Additionally, the rhizobacteria isolated from the PNAs also presented plant protection and health capabilities by producing compounds that reduce or stop the growth of phytopathogenic fungi, that is, they have potential for biological control, the benefits in seed germination are indicated (Gómez-Luna, et al 2012; Gómez-Luna, et al 2018; Gómez-Luna, et al 2020).

Conclusions

- Protected Natural Areas provide diverse services and resources for human beings and it is their responsibility to make a sustainable use of resources.
- A biotechnological and eco-friendly strategy is the use of beneficial microorganisms such as rhizobacteria, isolated from the soils of the PNA and applied to improve the plant production of the area and also plants of nutritional importance.

- With the application of rhizobacteria, the use of agrochemicals in plant production could be reduced.
- Rhizobacteria have the ability to be used as biofertilizer, biocontrol and biostimulant of the soil and with this regenerate or conserve the PNA and thus all its resources and services.
- These rhizobacteria that already exist naturally in the soils of the PNA.

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